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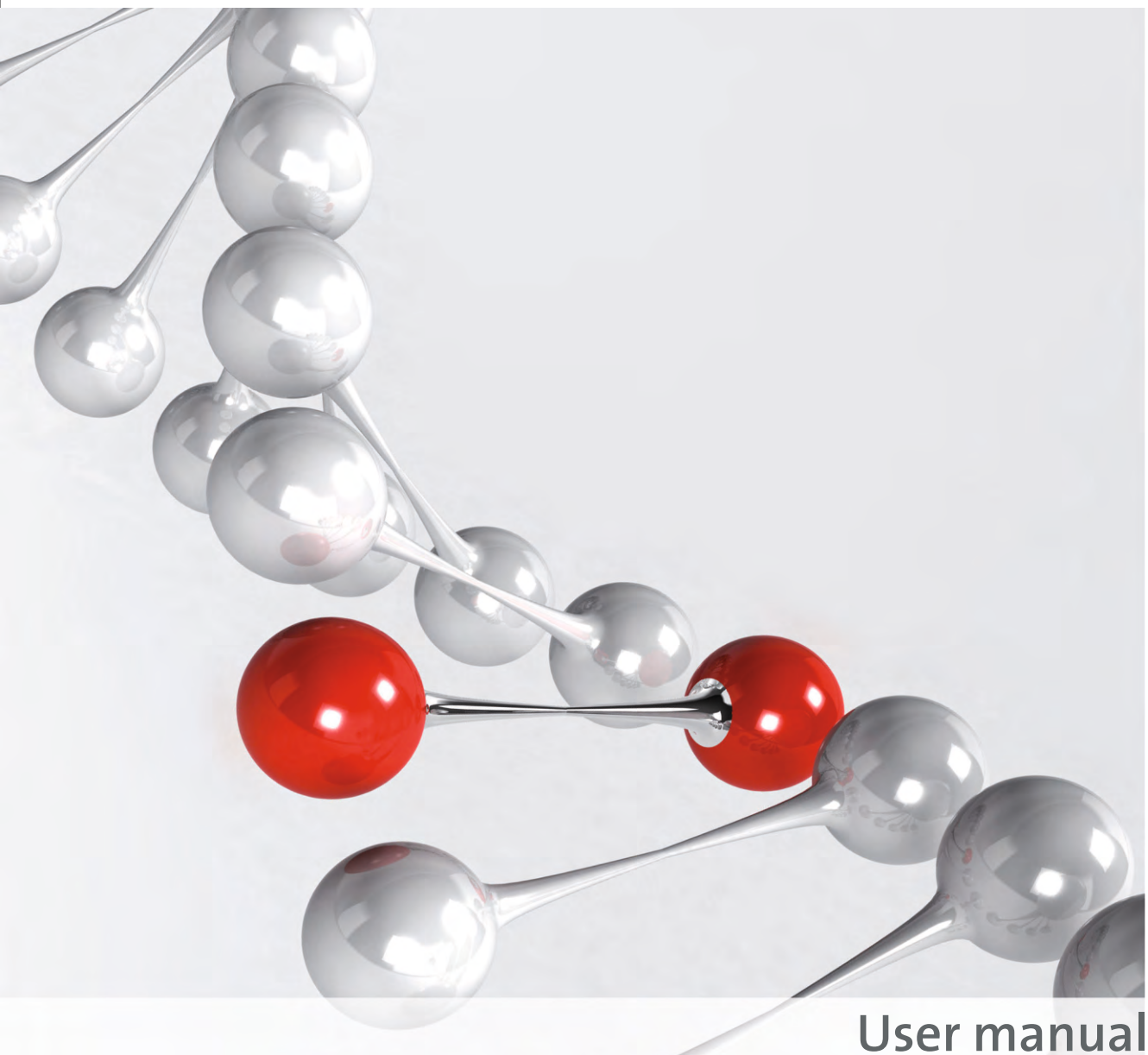

User manual
InviMag® Universal Kit/ KFDuo
for use on KingFisher® Duo, Thermo Fisher Scientific

for automated purification of DNA (genomic, bacterial, mitochondrial and viral) as well as viral RNA from 200 µl clinical samples with magnetic beads

REF 2450130x00 STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® Universal Kit/ KFDuo w/o plastic

The **InviMag® Universal Kit/ KFDuo** combines the advantages of the innovative InviMag® technology with easy handling of magnetic particles in combination with the KingFisher™ Duo robotic platform from Thermo Fisher Scientific for a very efficient and reliable isolation of nucleic acids with a high purity. The kit is the ideal tool for semi-automated isolation and purification genomic and bacterial DNA and/or viral DNA/RNA from up to 200 µl sample volume.

The interplay of the nucleic acid extraction and purification chemistry provided by the **InviMag® Universal Kit/ KFDuo** was intensely tested and validated.

The nucleic acid binding particles are characterized by a high surface area, uniform size distribution, good suspension stability and therefore are highly suitable for high-throughput processing.

Due to the high purity of the derived eluates, the isolated nucleic acids are ready-to-use in a broad spectrum of downstream applications or can alternatively be stored at -20°C/-80°C for subsequent use.

For research use only!

Trademarks: InviMag®, Invisorb®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag® and Invisorb® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of InviMag® Universal Kit/ KFDuo w/o plastic

Store the **SNAP Solution** and **Carrier RNA** at 2-8°C! Store dissolved **Proteinase K / Carrier RNA** in aliquots at -20 °C! Store all other kit components at room temperature (RT*)!

***Important:* The needed KFDuo plastic is not included in the kit (see ordering information at page 28)**

Component	8 x 12 preparations	40 x 12 preparations
Catalogue No.	2450130150	2450130250
Lysis Buffer HLT	30 ml	120 ml
Carrier RNA	2 pcs.	1 pcs.
Proteinase K	2 pcs.	1 pcs
SNAP Solution	2 x 1.1 ml	10.5 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 30 ml)	empty bottle (final volume 120 ml)
Wash Buffer HLT	90 ml (final volume 150 ml)	360 ml (final volume 600 ml)
Wash Buffer II	45 ml (final volume 150 ml)	180 ml (final volume 600 ml)
Wash Buffer M	30 ml (final volume 120 ml)	150 ml (final volume 600 ml)
Elution Buffer M	15 ml	60 ml
RNase free water	3 x 2 ml	2 x 15 ml
Sealing Foils	2	10
Manual	1	1
1.5 ml Receiver Tubes	2 x 50 pcs.	10 x 50 pcs.
Initial steps	Add 60 ml of abs. Isopropanol to the bottle **Wash Buffer HLT**, mix thoroughly and keep the bottle firmly closed! Add 90 ml of 96-100% ethanol to the bottle **Wash Buffer M**, mix thoroughly and keep the bottle firmly closed! Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle firmly closed! Resuspend the **Proteinase K** in 1.1 ml RNase-free water, mix thoroughly until completely dissolving and store at -20°C! Resuspend the **Carrier-RNA** in 1.2 ml RNase-free water. Mix thoroughly until completely dissolving. Add 30 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle	Add 240 ml of abs. Isopropanol to the bottle **Wash Buffer HLT**, mix thoroughly and keep the bottle firmly closed! Add 450 ml of 96–100% ethanol to the bottle **Wash Buffer M**, mix thoroughly and keep the bottle firmly closed! Add 420 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle firmly closed! Resuspend the **Carrier-RNA** in 15 ml RNase free water. Mix thoroughly until completely dissolving. Resuspend the **Proteinase K** in 10 ml RNase free water, mix thoroughly until completely dissolving and store at -20°C! Add 120 ml of 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle
Plastic to be supplied by user	**see order information**	
2.0 ml KF Deep Well Plate	8	40
KF-Duo 12-Tip Comb	8	40
KF-Duo Elution stripe	8	40

Symbols

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit contents of the **InviMag® Universal Kit/ KFDuo w/o plastic**, except **Carrier RNA** and **SNAP solution,** should be stored at room temperature* and are stable for at least 12 months at this condition.

Carrier RNA/ Proteinase K: Lyophilized **Carrier RNA** must be stored at 2-8°C. Dissolved **Carrier RNA** and **Proteinase K** must be stored at -20°C.

SNAP solution: The magnetic beads should be stored at 2-8°C.

Wash Buffer M/ Wash Buffer II / Wash Buffer HLT: Wash Buffers, charged with either ethanol or isopropanol, should be stored at room temperature and must be appropriately sealed. If any precipitates are visible within the provided solutions, solve them by carefully warming up to 30°C.

Room temperature (RT) is defined as range from 15-30°C.

Quality Control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Universal Kit/ KFDuo w/o plastic** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if a problem in the lot is detected, STRATEC Molecular will replace the product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® Universal Kit/ KFDuo w/o plastic** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® Universal Kit/ KFDuo w/o plastic** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany: +49-(0)30-9489-2901 / 2910
from abroad: +49-(0)30-9489-2903 / 2907 or contact your local distributor.

Intended use

The **InviMag® Universal Kit/ KFDuo w/o plastic** is designed for semi-automated rapid and economical preparation of nucleic acids (viral DNA/RNA, genomic DNA, bacterial DNA but not plasmid DNA) from 200 µl sample volumes like blood (EDTA/Citrate stabilized but not heparin), serum, plasma, cerebrospinal fluid, cell culture supernatant, cell-free body fluids, urine, supernatant from stool suspensions, rinsed liquid from swabs or bacterial suspensions, sputum, BAL using magnetic beads and the KingFisher™ Duo instrument from Thermo Fisher Scientific.

The whole process is based on the patented **InviMag®** technology, which relies on binding of the nucleic acids by magnetic particles. The procedure only requires minimal user interaction (prefilling of reagents), allowing safe handling of potentially infectious samples.

The provided isolation protocols and buffers are optimized to provide high yields and purities. However, for reproducible yields an appropriate sample storage and quick handling is essential. The purified viral DNA and/or RNA as well as bacterial or genomic DNA are ready-to-use for downstream analysis.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONAL WORKERS, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES.

The generated eluates can be used with any downstream application, employing enzymatic amplification or other modifications of DNA/RNA followed by signal detection or amplification. Any diagnostic results generated, using the sample preparation procedure in conjunction with any downstream diagnostic assay, should be interpreted with regards to other clinical or laboratory findings.

All utilities, except ethanol and isopropanol, required for preparation of nucleic acids are provided by the **InviMag® Universal Kit/ KFDuo w/o plastic.**

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is validated for e.g. viral DNA/RNA extraction from cell-free body fluids and rinsed liquids, specifically for human serum and plasma. Related applications will need a separate validation. Extraction of other than human DNA from blood or of total RNA has not been evaluated with this kit.

The included chemicals are only useable once.

Differing the starting material or flow trace may lead to inoperability. Therefore, neither a warranty nor guarantee in this case will be given, implied or expressed.

The user is responsible to validate the performance of the STRATEC Molecular products for any particular use. STRATEC Molecular does not provide validations of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory. The laboratory must be validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and plastic parts are for laboratory use only. They should be stored in the laboratory and must not be used for other purposes than intended.

The kit components are not suitable for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles! Avoid direct skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, WEAR GLOVES, AND PROTECTIVE GOGGLES when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Universal Kit/ KFDuo w/o plastic** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered and handled as infectious and discarded accordingly to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Universal Kit/ KFDuo w/o plastic** to which they apply are listed below as follows:

Lysis Buffer HLT

contains guanidine-hydrochloride

warning

H302-315-319, P280-505+351+338

Proteinase K

danger

H315-319-334-335 P280-305-351-338-310-405

Wash Buffer HLT

contains guanidine-hydrochloride

warning

H302-315-319, P280-505+351+338

H302	Harmful if swallowed.
H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses and continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
inside of USA: 1 – 800 – 535 – 5053

Product characteristics of the InviMag® Universal Kit/ KFDuo w/o plastic

Starting Material	Yield	Time for preparation
up to 200 µl cell free body fluids like serum, plasma or liquor, etc. up to 200 µl rinsed liquid from swabs up to 200 µl cell culture supernatants up to 200 µl blood (EDTA / citrate stabilized, but not heparin) 1x 10^6 mammalian cells 10 mg tissue sample	Depends on sample (storage and source) ***Note:*** The added Carrier-RNA will account for most of the eluted nucleic acids. Quantitative (RT)-PCR is recommended for determination of the viral RNA or DNA yield	about 60 min

The **InviMag® Universal Kit/ KFDuo w/o plastic** is the ideal tool for an efficient and semi-automated extraction of genomic and/or bacterial DNA and viral DNA/RNA from different sample sources.

The nucleic acid isolation process is based on the interaction of nucleic acids with silica coated magnetic particles in presence of adjusted buffer conditions.

The DNA/ RNA purification procedure is performed with minimal user intervention, except the initial loading of the system and plate preparation. This allows safe handling of potentially infectious samples. Sample cross-contamination and reagent cross-over is effectively eliminated.

The KingFisher instrument uses magnetic rods to transport the DNA/RNA-binding magnetic particles through the various extraction phases: lysis-binding-washing-elution. The automated purification process results in a fast, reliable and robust technique.

After a sample specific lysis, optimal binding conditions are adjusted. The genomic DNA and/or viral DNA/RNA will bind to the added magnetic particles and is separated from the solution by magnetic rods controlled by the KingFisher system. Subsequent to three washing steps the nucleic acids are finally eluted from the beads.

Due to the high purity, the eluted nucleic acids are ready-to-use in a broad panel of downstream applications like:

- PCR*, real-time PCR
- Restriction Enzyme Digestion
- HLA Typing
- Southern Blot

For the isolation of DNA from single blood samples, STRATEC Molecular offers the Invisorb® Spin Blood Mini Kit or for 8–96 samples, the Invisorb® Blood Mini 96 HTS Kits for use on a centrifuge, vacuum manifold or other robotic stations.

For the isolation of viral RNA, DNA or both, STRATEC Molecular offers a series of spin kits as well as HTS kits for use on centrifuge, vacuum manifold, or for a walk-away automated isolation on other robotic stations as well as magnetic bead based kits (see page 28).

* The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Sampling and storage of starting material

For reproducible and high yields, the correct sample storage is essential. Yields may vary from sample to sample depending on factors such as health of the donor, sample age, kind of sample, transport and storage conditions. Best results are obtained with fresh material.

Cultivated bacteria or bacterial suspension(s):
Bacteria have to be pelleted after cultivation and need to be resuspended at defined conditions.

Swabs, Saliva: The protocol works with fresh saliva, prepared swabs as well as with dried swabs. The protocol is not validated for the isolation of DNA from swabs using storage buffers from other providers.
As long as the samples are not shock frosted in liquid nitrogen or incubated with RNase inhibitors or denaturing reagents, the viral RNA is not secured. Therefore, it is essential that samples are immediately flash frozen subsequent to harvesting by using liquid nitrogen and storage at -80°C. Viral RNA in deep frozen samples is stable for months. Viral RNA purification should be processed as soon as possible.

Urine: The bacteria should be pelleted while the supernatant is discarded (urea contaminations may inhibit PCR reactions).For some applications fresh urine can be used directly.

Blood: Best results are obtained using fresh blood samples. Blood samples (stabilized with EDTA or citrate but **not** heparin) can be stored at room temperature (18-25°C) for 2-3 hours. For short-term storage (up to 24 h) samples should be stored at 4-8°C. For long-term storage, we recommend to freeze the samples at -20°C or -80°C. Avoid multiple thawing and freezing cycles of the sample(s) before isolating the DNA/RNA because this may lead to degraded DNA.

Stool samples: Best results are obtained with fresh material. Stool samples contain DNases and RNases which realize quickly DNA and RNA digestion and degradation. The sample may be stored frozen at – 80°C.

Serum and plasma (and other cell-free body fluids): After collection and centrifugation, serum or plasma derived from blood (treated with anticoagulants like EDTA or citrate, but not with heparin), synovial fluid samples or other cell free body fluids and rinsed liquids from swabs can be used for extraction. For short-term storage samples can be kept on ice for 1-2 hours or for up to 24 hours samples may be stored at -20°C. For long-term storage, we recommend freezing samples in aliquots at –80°C. Frozen plasma or serum samples must not be thawed more than once. Multiple thawing and freezing cycles before isolating the viral DNA/RNA should be avoided because this may lead to denaturation/precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. In addition, cryoprecipitate formed during freeze-thawing cycles can cause problems. If cryoprecipitate are visible, pellet them by centrifugation at 6.800 x *g* for 3 minutes. The cleared supernatant should be aspirated, without disturbing the pellet and processed immediately. This step will not reduce viral titers.

Cell culture supernatants: Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C after obtaining the cell culture supernatant. Repeated freezing and thawing cycles of stored samples will negatively influence the sensitivity.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the chapter “Intended Use” are processed or if the sample preparation protocols are changed or modified.

Principle and procedure

The **InviMag® Universal Kit/ KFDuo w/o plastic** procedure comprises following steps:

- Sample preparation (if required)
- Lysis at different temperatures
- Adjustment of binding conditions
- Binding of the nucleic acids to magnetic beads
- Washing of the bead bound nucleic acids and evaporation of ethanol
- Elution of nucleic acids

Procedure

Bacteria must be cultivated at special conditions. An aliquot of the bacterial suspension is used to form a pellet by centrifugation at high speed for 5 min. The supernatant is discarded.

Pretreatment: Please check the corresponding section in the protocol.

Lysis
Samples are lysed at elevated temperatures in the presence of **Lysis Buffer HLT** and **Proteinase K** (**Lysozyme if required)** to break bacterial and viral cell walls and to digest proteins.

The addition of **Carrier-RNA** is required for the enhancement and stabilization of viral DNA/RNA recovery. Due to this, it is even possible to purify very small amounts of viral DNA/RNA molecules.

Binding of the nucleic acids
After addition of **Binding Solution** to adjust optimal binding conditions, the nucleic acids are bound by the simultaneously added magnetic beads (**SNAP Solution**).

Removing residual contaminants
Contaminants are efficiently removed during the washing process using **Wash Buffer HLT**, **Wash Buffer M** and **Wash Buffer II** while the nucleic acids remain bound to the beads.

Elution
The nucleic acids are finally eluted in **Elution Buffer M** and are ready-to-use in different subsequent downstream applications, e.g. PCR amplification, digestion with restriction enzymes, Southern hybridizations, HLA typing, etc.

Yield and quality of genomic DNA derived from Blood

The amount of purified DNA/RNA in the **InviMag® Universal Kit/ KFDuo** procedure from whole blood depends on the leucocytes content, the sample source, transport, storage and age.

Typically, a volume of 200 µl whole blood from a healthy individual with an elevated white blood cell content - ranging from $3x10^6$ to $1x10^7$ cells / ml - will yield 2-6 µg of genomic DNA. If a whole blood sample is mixed with anticoagulant containing buffer solutions the overall leukocyte concentration decreases and the yield is reduced.

Please keep in mind that the added Carrier-RNA will falsify the real genomic DNA content in photometric measurements.

Yield and quality of viral nucleic acids

The amount of purified nucleic acids in the **InviMag® Universal Kit/ KFDuo w/o plastic** procedure depends on the sample type, virus titer, sample source, transport, storage and age.

Yield and quality of the isolated viral nucleic acids is suitable for any detection system. Diagnostic tests should be performed accordingly to the manufacturers' specifications.

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Eluates derived by this kit will contain Carrier-RNA, which will greatly exceed the amount of the isolated viral NA.

Yields of viral nucleic acids isolated from biological samples are usually low concentrated and therefore almost impossible to determine photometrically.*

* Keep in mind that the Carrier-RNA (5 µg per 200 µl sample) will account for most of the present RNA.

The kit is suitable for downstream analysis with NAT techniques, for examples qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed accordingly to the manufacturer's instructions.

Quantitative RT-PCR is recommended for determination of viral RNA yield.

** In Gel Electrophoresis and in Capillary Electrophoresis, RNA extracted with the provided kit looks like degraded cause the kit contains Carrier RNA, this is poly A RNA in fragments of 100 up to 1000 bases. The kit is not dedicated for applications using this kind of analysis.*

Important notes

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages and correct quantities. If there are any unconformities, notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 6). Do not use damaged kit components, because their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. We recommend the use of aerosol-barrier pipet tips to avoid cross-contaminations.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- We recommend working under laminar air-flow until the samples are lysed to minimize the risk of infections from potentially infectious material.

This kit should only be used by trained personnel.

Preparing reagents and buffers

8 x 12 extractions:
Add 60 ml of abs. Isopropanol to the bottle **Wash Buffer HLT**, mix thoroughly and keep the bottle firmly closed!
Add 90 ml of 96-100% ethanol to the bottle **Wash Buffer M**, mix thoroughly and keep the bottle firmly closed!
Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle firmly closed!
Resuspend the **Proteinase K** in 1.1 ml RNase-free water, mix thoroughly until completely dissolving and store at -20°C!
Resuspend the **Carrier-RNA** in 1.2 ml RNase-free water. Mix thoroughly until completely dissolving.
Add 30 ml of 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle
40 x 12 extractions:
Add 240 ml of abs. Isopropanol to the bottle **Wash Buffer HLT**, mix thoroughly and keep the bottle firmly closed!
Add 450 ml of 96–100% ethanol to the bottle **Wash Buffer M**, mix thoroughly and keep the bottle firmly closed!
Add 420 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle firmly closed!
Resuspend the **Carrier-RNA** in 15 ml RNase free water. Mix thoroughly until completely dissolving.
Resuspend the **Proteinase K** in 10 ml RNase free water, mix thoroughly until completely dissolving and store at -20°C!
Add 120 ml of 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle

Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information's, please consult the appropriate material safety data sheets (MSDS) on our webpage: **www.stratec.com**.

- Microcentrifuge ≥ 9.300 x *g* (≥ 10.000 rpm), optional*
- Ethanol (96-100%)
- 1.5 ml reaction tubes, optional
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipet with tips (we highly recommend to use filter tips only)
- 15 or 50 ml reaction tubes, optional
- Isopropanol*

The **InviMag® Universal Kit/ KFDuo w/o plastic** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth.**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Important indications

Preparing RNA
When preparing RNA, work quickly during the manual steps of the procedure. Special care should be taken to avoid contaminations of reagents with DNases/ RNases.

Storing samples
Frozen serum or plasma samples should not be thawed more than once. Repeated freezing and thawing cycles may lead to denaturation and precipitation of proteins, resulting in reduced titers and yields of nucleic acids.

Carrier-RNA
Carrier-RNA serves two purposes: It enhances the binding of nucleic acids to the beads, especially if there are only very few target molecules in the sample. Furthermore, the addition of Carrier-RNA reduces the chance of nucleic acid degradation in the rare event that RNase or DNase molecules are not inactivated completely by the Lysis Buffer.

Internal Controls
The use of an internal control is recommended when using the **InviMag® Universal Kit/ KFDuo w/o plastic** in combination with diagnostic amplification systems. Internal controls should be added directly to the lysis mixture during the pause step when the Binding Solution and beads are added. Do not add any controls to the sample or to lysis mixture until the lysis step is complete. In rare cases the controls may be degraded by DNases/RNases present in the sample.
Alternatively, internal controls can be prepared directly in the Carrier-RNA tube. In that case, the amount of added water to the Carrier-RNA has to be reduced. Do not exceed the final filling volume of the Carrier-RNA tube.

Scheme of the InviMag® Universal Kit/ KFDuo w/o plastic

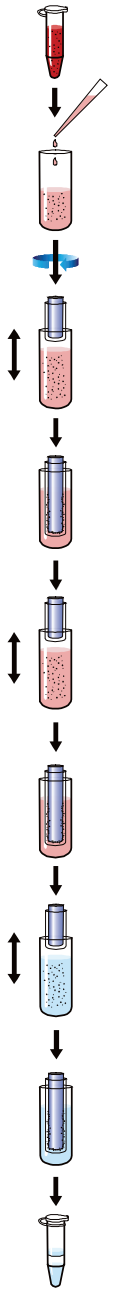
Please read protocols prior the start of the preparation carefully!

Transfer 200 µl **Lysis Buffer HLT** and 200 µl **sample** into an unused cavity of row 1 of the 2 ml Deep Well Plate (refers as "Lysis Plate"). Add 20 µl Proteinase K and 20 µl Carrier-RNA (optional for genomic / bacterial DNA preparations). For bacterial DNA preparation, 20 µl Lysozyme (not provided, stock solution 10 mg/ml) must be added. Continue with the respective lysis protocol (see pages 14).

Prefill the Working Plate (2 ml DWP) and Elution stripe with required buffers and appropriate volumes.

Working Plate (2 ml DWP)

Tip: (Row 2):	Insert the KF-Duo 12-Tip Comb in row 2 of the Working Plate
Lysis: (Row 1)	See "lysis procedures" (page14) for respective protocol
Wash 1: (Row 3):	Add 900 µl **Wash Buffer HLT** to row 3 of the Working Plate
Wash 2: (Row 4)	Add 900 µl **Wash Buffer M** to row 4 of the Working Plate
Wash 3: (Row 5)	Add 1000 µl **Wash Buffer II** to row 5 of the Working Plate
Elution stripe:	Add 100 µl **Elution Buffer M** to the Elution stripe



Please carefully read the protocols prior to the start of the preparation procedure!

The following steps are performed on the KingFisher instrument:

Lysis of the sample

After lysis, a pause step occurs and **230 µl Binding Solution** and **20 µl SNAP Solution** have to be added to each lysate in row 1 of the Working Plate.

Important: *If an internal extraction control should be used, please add it to the reaction mixture at this step.*

Nucleic acids bind to magnetic particles

Washing of the particle fixed nucleic acids

Magnetic separation

Elution of nucleic acids

Magnetic Separation

Pure nucleic acids

Lysis procedures

For easier handling we recommend to prepare a master mix consisting of Lysis Buffer HLT, Proteinase K and if required Carrier-RNA. When preparing the **Master Mix** it is recommended to prepare a volume of 5% greater than that required.

Protocol 1: Simultaneous isolation of nucleic acids (viral DNA/RNA) from cell free body fluids or of DNA from blood (genomic DNA)

Please read the protocols carefully prior to the start of the preparation procedure!

Important Note: *The protocol is optimized for a sample volume of 200 µl. For smaller samples volumes than 200 µl please adjust to a total volume of 200 µl with ddH_2O or PBS.*

Transfer 200 µl of sample into a free cavity of row 1 of the Working Plate and add 200 µl Lysis Buffer HLT, 20 µl Proteinase K and 20 µl Carrier-RNA. If genomic DNA shall be prepared, the addition of Carrier-RNA is optional.

Prefill the remaining rows of the Working Plate with the required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 18).

Protocol 2: Simultaneous isolation of nucleic acids (DNA/RNA) from swab material

Please read the protocols carefully prior to the start of the preparation procedure!

2.1 Use of swabs

Transfer 200 µl Lysis Buffer HLT into a cavity of row 1 of the Working Plate and add 200 µl distilled water, 20 µl Proteinase K and 20 µl Carrier-RNA. If bacterial DNA is processed, 20 µl Lysozyme (not provided, stock solution 10 mg/ml) should be added. If genomic or bacterial DNA is processed, the addition of Carrier-RNA is optional.

Insert the swab into the cavity of row 1 of the Working Plate. Incubate for 5-10 min at RT and stir occasionally. After incubation, remove the swab and squeeze it out inside the corresponding cavity to remove residual liquid and then discard the swab.

Prefill the remaining rows of the Working Plate with the required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 18).

2.2. Usage of rinsed liquid from swab

a) the sample <u>will also be used</u> for cultivation

Cut off the relevant part of the swab and transfer it into an RNase/DNAse-free 2 ml tube. Add 300 µl physiological saline solutions to the swab and vortex intensely for 2-3 min and incubate for 10 min at RT. Use an aliquot for cultivation.

For the preparation of bacterial DNA, 20 µl Lysozyme (stock 10 mg/ml, not provided) have to be added for an improved lysis. Add the Lysozyme directly to row 1 of the Working Plate before adding samples or other reagents.

Transfer 200 µl of the rinsed liquid into a free cavity of row 1 of the Working Plate and add 20 µl Proteinase K and 20 µl Carrier-RNA. If genomic or bacterial DNA is prepared, the addition of Carrier-RNA is optional. Prefill the remaining rows of the Working Plate with the required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 18).

b) the sample will not be used for cultivation

Cut off the relevant part of the swab and transfer it into an RNase- and DNAse-free 2 ml tube. Add 300 µl RNase-free water to the swab and vortex intensely for 3 min. Optional, incubate for 3 min at 95°C.

For the preparation of bacterial DNA, 20 µl Lysozyme (stock 10 mg/ml, not provided) should be used for an improved lysis. Add the Lysozyme directly to row 1 of the Working Plate before adding samples or other reagents.

Transfer 200 µl of the rinsed liquid into a cavity of row 1 of the Working Plate and add 20 µl Proteinase K and 20 µl Carrier-RNA. If genomic or bacterial DNA is prepared, the addition of Carrier-RNA is optional. Prefill all remaining rows of the Working Plate with the required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 18).

Protocol 3: Simultaneous isolation of nucleic acids (DNA and RNA) from tissue biopsies

Please read the protocols carefully prior to the start of the preparation procedure!

Transfer 1-10 mg from the tissue biopsy sample into a cavity of row 1 of the Working Plate and add 200 µl distilled water, 200 µl Lysis Buffer HLT, 20 µl Carrier-RNA and 20 µl Proteinase K. If genomic DNA is prepared, the addition of Carrier-RNA is optional. For the preparation of bacterial DNA, 20 µl Lysozyme (stock 10 mg/ml, not provided) should be added for an improved lysis.

Prefill all remaining rows of the Working Plate with the required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 18).

Protocol 4: Isolation of DNA from bacteria pellets (up to 1x 10^9 bacterial cells) or from some ml of non-viscous tracheal secrete of BAL

Please read the protocols carefully prior to the start of the preparation procedure!

For the preparation of bacterial DNA, we recommend using 20 µl Lysozyme (stock 10 mg/ml, not provided) for an improved lysis. Add the Lysozyme directly to row 1 of the Working Plate before adding samples or other reagents.

For sample preparation use an aliquot from the bacterial culture and centrifuge the sample at 9.300 x *g* (10.000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet.

Resuspend the bacterial pellet in 200 µl distilled water or PBS and transfer the sample into a cavity of row 1 of the Working Plate. Add 200 µl Lysis Buffer HLT and 20 µl Proteinase K.

Prefill all remaining rows of the Working Plate with the required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 18).

Protocol 5: Simultaneous isolation of total nucleic acids from sputum

Please read the protocols carefully prior to the start of the preparation procedure!

For the preparation of bacterial DNA, we recommend using 20 µl Lysozyme (stock 10 mg/ml, not provided) for an improved lysis. Add the Lysozyme directly to row 1 of the Working Plate before adding samples or other reagents.

Transfer 150 µl of the sputum sample into an RNase/DNAse-free tube and add 150 µl NAC Buffer (order number: 1033221100) or saturated acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:1).

Incubate the mixture for 10 min at 95°C to reduce the viscosity and transfer 200 µl from the mixture into a free cavity of row 1 of the Working Plate.

Add 200 µl Lysis Buffer HLT, 20 µl Carrier-RNA and 20 µl Proteinase K. If genomic or bacterial DNA is processed, the addition of Carrier-RNA is optional.

Prefill all remaining rows of the Working Plate with the required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 18).

Protocol 6: Simultaneous isolation of viral nucleic acids (DNA and RNA) from slimy tracheal secretes or BAL

Please read the protocols carefully prior to the start of the preparation procedure!

Important: For the preparation of bacterial DNA, we recommend using 20 µl Lysozyme (stock 10 mg/ml, not provided) for an improved lysis. Add the Lysozyme directly to row 1 of the 2 ml DWP (refers as “Working Plate”) before adding samples or other reagents.

Non viscous samples:

Transfer 1 ml of tracheal secret or BAL into an RNase/DNAse-free tube and centrifuge at 9.300 x *g* (10.000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet: Resuspend the bacterial pellet in 200 µl distilled water or PBS and transfer the sample into row 1 of the Working Plate. Add 200 µl Lysis Buffer HLT, 20 µl Carrier-RNA and 20 µl Proteinase K.

Prefill all remaining rows of the Working Plate with the required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 18).

Viscous sample

Transfer 1 ml of tracheal secrete or BAL into a RNase- and DNAse-free tube and add 1 ml NAC Buffer (order number: 1033221100) or saturated acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:1).

Incubate the mixture for 10 min at 95°C to reduce the viscosity and centrifuge at 9.300 x *g* (10.000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet directly.

Resuspend the bacterial pellet in 200 µl distilled water or PBS and transfer to a free cavity of row 1 of the Working Plate. Add 200 µl Lysis Buffer HLT, 20 µl Carrier-RNA and 20 µl Proteinase K

Prefill all remaining rows of the Working Plate with the required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 18).

Protocol 7: Simultaneous isolation of viral nucleic acids from stool samples

Please read the protocols carefully prior to the start of the preparation procedure!

Transfer 100 µl of stool sample into a 2 ml tube and dilute the sample 1:10 with RNase-free water. Vortex the sample for 30 s followed by a 1 min centrifugation step at 12.000 x *g* (13.000 rpm).

Transfer 200 µl of viral containing supernatant to row 1 of the Working Plate. Add 200 µl Lysis Buffer HLT, 20 µl Carrier-RNA and 20 µl Proteinase K to each sample containing cavity.

Prefill the remaining rows of the Working Plate with the required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 18).

Protocol 8: Isolation of bacterial DNA from stool samples

Please read the protocols carefully prior to the start of the preparation procedure!

Important: For the preparation of bacterial DNA, we recommend using 20 µl Lysozyme (stock 10 mg/ml, not provided) for an improved lysis. Add the Lysozyme directly to row 1 of the Working Plate before adding samples or other reagents.

Transfer 100 µl stool sample into a 2 ml tube and add 300 µl RNase-free water. Vortex the sample for 30 s followed by a 30 s centrifugation step at 3.000 rpm. (1.000 x *g*)

Transfer 200 µl of the bacteria containing supernatant to a cavity of row 1 of the Working Plate. Add 200 µl Lysis Buffer HLT and 20 µl Proteinase K.

Prefill the remaining rows of the Working Plate with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 18).

Starting a Run

I. Preliminary Steps to process the sample onto the KingFisher System

Important: *For working with the KFDuo instrument, please carefully read the manufacturer´s manual before using the system for the first time!*

1. Switch on the KF-Duo instrument using the power switch.

2. Prefill the Working Plate and Elution stripe with the required buffers and appropriate volumes.

Important: *Mix the bottle with the **SNAP Solution** by vigorously vortexing before usage!*

Working Plate:

Lysis: See the corresponding isolation protocol (chapter “Lysis procedures”, page 14)

Tip: Place the KF-Duo 12-Tip Comb for DW magnets into *row 2* of the Working Plate.

Wash 1: Add 900 µl **Wash Buffer HLT** to *row 3* of the Working Plate

Wash 2: Add 900 µl **Wash Buffer M** to *row 4* of the Working Plate

Wash 3: Add 1000 µl **Wash Buffer II** to *row 5* of the Working Plate

Elution stripe: Add 100 µl **Elution Buffer M** to the separate elution stripe

3. Choose the assay ”**InviMag_Universal_KF-Duo”** on the display of the instrument and press the “START” button.
4. Insert the prefilled Working Plate and Elution stripe onto the corresponding positions of the KingFisher Duo surface. Press the “START” button to initialize the assay.
5. After the lysis steps, a pause will occur and 230 µl **Binding Solution** and 20 µl **SNAP Solution** have to be added to each used cavity of row 1 of the Working Plate. If extraction controls should be used please add them at this step too. Alternatively, the internal control can be added to the Carrier-RNA tube (see page 12).
6. Reinsert the Working Plate into the instrument (watch out for correct plate orientation) and continue the run by pressing the “START” button. The instrument will now finish the purification process without any further user interaction.

II. The following steps run automatically on the KingFisher System

Specific sample preparation

1. Lysis
Lysis is performed at elevated temperature for 15 min. After lysis, the instrument will be paused and the user has to add **230 µl Binding Solution** and **20 µl SNAP Solution** (magnetic beads). Internal extraction controls should be added during the pause step too or can alternatively be added to the Carrier-RNA (see page 12).

2. **Binding of the nucleic acids**
Binding step for 5 min. SNAP separation. Transportation of the SNAP bound nucleic acids into Wash 1.

3. **First Washing Step**
Sample washing for 5 min. SNAP separation. Transportation of the SNAP bound nucleic acids into the Wash 2.

4. **Second Washing Step**
Sample washing for 4 min. SNAP separation. Transportation of the SNAP bound nucleic acids into the Wash 3.

5. Third Washing and Drying Step
Sample washing for 3 min. SNAP separation. Drying the SNAP bound nucleic acids outside of the plate for 5 min. Transportation of the SNAP into the Elution stripe.

6. **Elution of the nucleic acids**
Incubation of the SNAP bound nucleic acids into the Elution stripe for 10 min by mixing at elevated temperature. SNAP separation. The SNAPs without the bound nucleic acids are afterwards automatically discarded into the wells of Wash 3 (row 5) of the Working Plate (disposal).

Important Notes: *After finishing the extraction protocol, the Elution stripe contains the extracted nucleic acids. Store the nucleic acids at adequate conditions. We recommend transferring the extracted nucleic acids into 1.5 ml Receiver Tubes (provided) and store them at -20°C or –80°C.*

If the extracted nucleic acids contain carry-over of magnetic particles, transfer them into a 1.5 ml reaction tube, centrifuge at max. speed for 1 min and then transfer the nucleic acids containing supernatant into the provided Receiver Tubes.

The eluted nucleic acids are ready-to-use in different downstream applications.

For self-programming of the KFDuo instrument

Reagent info

A (Lysis/Binding)		Working Plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Sample	200	-	Sample
Lysis Buffer HLT	200	-	Reagent
Proteinase K	20	-	Reagent
Carrier-RNA (if required)	20	-	Reagent
Lysozyme (if required)	20	-	Reagent

B (Tip Comb)		Working Plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

C (Wash 1)		Working Plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer HLT	900	-	Reagent

D (Wash 2)		Working Plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer M	900	-	Sample

E (Wash 3)		Working Plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	1000	-	Reagent

F		Working Plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

G		Working Plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

H		Working Plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

A (Elution)		Elution Stripe	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer M	100	-	Reagent

Dispensed reagents

A (Lysis/Binding)	Working Plate

Steps data

Tip1		KingFisher Duo 12 tip comb	
	Pick-Up	Working Plate	(B) - Tip Comb
	Lysis Step	Working Plate	(A) - Lysis/Binding
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:15:00, Medium
		Heating temperature [°C]	75
	End of step	Postmix	No
		Collect beads	No
		Post-temperature	No
	Adjust Binding	Working Plate	(A) - Lysis/Binding
		Message	Add Isopropanol + SNAPs
		Dispensing volume [µl]	250
	Reagent(s)	Name	Isopropanol
		Volume [µl]	230
		Name	SNAP Solution
		Volume [µl]	20
	Binding Step	Working Plate	(A) - Lysis/Binding
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
		Post-temperature	No
	Washing Step 1	Working Plate	(C) - Wash 1
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
		Post-temperature	No
	Washing Step 2	Working Plate	(D) - Wash 2
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:04:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
		Post-temperature	No

	Washing Step 3	Working Plate	(E) - Wash 3
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:03:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
		Post-temperature	No
	Drying Step	Working Plate	(E) - Wash 3
		Dry time	00:05:00
		Tip position	Outside well / tube
	Elution Step	Elution Stripe	(A) - Elution
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	65
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	10
		Post-temperature	No
	Bead Removal	Working Plate	(E) - Wash 3
		Release time, speed	00:00:30, Fast
	Leave	Working Plate	(B) - Tip Comb

Troubleshooting

Problem	Probable cause	Comments and suggestions
Low amount of extracted DNA	Insufficient lysis	Increase lysis time, but prevent too long lysis time because this also decreases the yield Reduce amount of starting material
	Incomplete elution	Increase the volume of **Elution Buffer M.** ensure that the **Elution Buffer M** is transferred into the right position; change the modified volume in the provided assay file too (max 130 µl)
	Low amount of SNAP Solution	Mix **SNAP Solution** vigorously before use
Low concentration of extracted DNA	Too much Elution Buffer	Elute the DNA in a lower volume of **Elution Buffer M**. Change the modified volume in the run file too.
	Incorrect storage of starting material	Ensure that the storage of starting material was correct. Avoid repeated thawing and freezing cycles of the sample material
	Incorrect Wash Buffers	Ensure, that the correct amount of ethanol or isopropanol was added to the **Wash Buffers** and they are stored correctly
Degraded DNA	Incorrect storage of starting material	Ensure that the storage of starting material was correct
	Old material	Ensure that the starting material is stored at appropriate conditions (–20°C/-80°C) avoid multiple thawing and freezing cycles of the material
DNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	No PCR result for genomic DNA	Due to the very gentle isolation procedure it may happen that isolated genomic DNA forms a ball. To overcome this, the primary PCR denaturation step at 95°C should be prolonged to 5 min
	Ethanol carryover during elution	Increase drying time for removal of ethanol in the assay file
	Salt carry over during elution	Check the **Wash Buffers** for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C Ensure that the **Wash Buffers** are equilibrated at room temperature
Eluted DNA is brownish colored	Small part of the magnetic particles are left in the elution	Centrifuge at full speed for 1 min and transfer supernatant to a new tube

Problem	Probable cause	Comments and suggestions
Low amount of extracted RNA	Insufficient lysis	Increase lyses time, but prevent too long lyses time because this decrease the yield Reduce amount of starting material
	Incomplete elution	Use a higher volume of **Elution Buffer M**. Ensure you pipet the **Elution Buffer M** with the correct volume to the right position. Change the modified volume in the provided assay file too (max 130 µl)
	Low amount of SNAP Solution	Mix **SNAP Solution** thoroughly before pipetting to the Deep Well Plate
Low concentration of extracted RNA	Too much Elution Buffer R	Elute the RNA with lower volume of **Elution Buffer M.** Change the modified volume in the run file too
	Incorrect storage of starting material	Ensure that the storage of starting material was correct avoid repeated thawing and freezing cycles of the sample material
	Incorrect Wash Buffers	Ensure that the correct amount of ethanol or isopropanol was added to the **Wash Buffers**
Degraded RNA	Incorrect storage of starting material	ensure that the storage of starting material was correctly Avoid multiple thawing and freezing cyclesof the sample material
	Old material	Ensure that the starting material is fresh or stored at appropriate conditions (-20°C / -80 C) Avoid multiple thawing and freezing of the material
RNA does not perform well in downstream-applications (e.g. real-time RT-PCR or RT-PCR)	Ethanol carryover during elution	increase drying time for removal of ethanol in the assay file
	Salt carryover during elution	Check the **Wash Buffers** for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C Ensure that the **Wash Buffers** are equilibrated at room temperature
Eluted RNA is brownish colored	Small parts of the magnetic particles are left in the elution	Centrifuge at full speed for 1 min and transfer supernatant into a new tube

Appendix

KingFisher BindIt Software 3.2 or higher versions

BindIt software 3.2 or higher versions was used to create the assay file(s) for the KFmL, KF96/KFflex96 or KFDuo instruments. The provided assay file(s) can either be transferred onto the corresponding workstation(s) or be started directly from within the BindIt software after assay import. Please keep in mind, that assay(s) run from within the BindIt software are not stored in the workstation memory.

Important: *Be advised that BindIt SW 3.2 or higher versions use a new unique file extension. Therefore, it is not possible to import assay files created with BindIt 3.2 or higher versions into older BindIt software versions! Please ask your local Thermo Scientific distributor for a software update.*

Note*:* *When creating assay files for usage with KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.2 or higher versions for assay development because this software version includes the correct adjustments for the microtiter plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 / KF-Duo workstations to ensure the best purification result.*

Minimum system requirements for BindIt Software 3.2 or higher versions

PC requirements	
Supported operating systems	MS Windows XP Pro with SP3, Windows Vista SP2, Windows 7
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 1 GHz
Memory	1 GB RAM
Serial ports available	1 (for KFmL connection)
USB ports available	1 (for KF96 / KFflex96 / KFDuo connection)
Pointing device	Mouse or equivalent is required
CD-ROM drive	1
Monitor / color settings	XVGA monitor with at least 1024x768 resolution and at least a 16-bit color environment

If the actual Windows Service Packs are not installed on the corresponding lab computer, they can be downloaded from the Microsoft web pages: http://www.microsoft.com/.

General notes on handling DNA

Nature of DNA
The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure it will work well in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR, and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA
For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -80°C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA
Store DNA at 2-8°C. Storage of genomic DNA at -20°C may cause shearing, particularly if the DNA is exposed to repeated freezing and thawing cycles.

Drying, dissolving and pipetting DNA
Avoid overdrying genomic DNA after ethanol precipitation. It is better to air-dry DNA than to use a vacuum, although vacuum drying can be used with caution.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively incubate the DNA in buffer overnight at 2-8°C. Minimize vortexing of genomic DNA because this can cause shearing.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings can cause shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA. Regular pipette tips pose no problem for plasmid and other small DNA.

Quantification
Quantification of DNA and RNA from this assay must be done by means of qPCR or Reverse Transcriptase qPCR. All other methods will be disturbed by the included Carrier-RNA as well as DNA or RNA which will be co-purified.

General notes on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum. Avoid handling bacterial cultures, cell cultures, or other biological sources of RNases in the same lab where the RNA purification is carried out.

All glassware must be RNase free. Therefore, the glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for four or more hours before use. Autoclaving only will not completely inactivate many RNases. Oven baking will inactivate RNases and ensure that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. It is possible to clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware must react 12 hours at 37°C and should then be autoclaved or heated to 100°C for 15 min to inactivate residual DEPC.

- **Electrophoresis tanks** should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase free water, rinsed with ethanol and allowed to air-dry.
- **Non-disposable plastic ware** must be treated before use to ensure that it is RNase-free. Plastics should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. It is possible to use chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All solutions must be prepared with DEPC-treated RNase-free water.
- Change gloves frequently and keep tubes closed.
- When handling RNA, reduce the preparation time as much as possible.
- Only use sterile disposable polypropylene tubes throughout the procedure.
- Always keep RNA samples on ice.

This kit should only be used by personnel trained in laboratory practice.

Storage of RNA

Purified viral RNA can be stored at -80°C and is stable for several years at this condition.

Quantification

Quantification of DNA and RNA from this assay must be done by means of qPCR or Reverse Transcriptase qPCR. All other methods will be disturbed by the included Carrier-RNA as well as DNA or RNA which is co-purified.

Ordering information

Product	Package Size	Catalogue No.
InviMag® Universal Kit/ KFDuo w/o plastic	8 x 12 preparations	2450130150
InviMag® Universal Kit/ KFDuo w/o plastic	40 x 12 preparations	2450130250

KingFisher Duo and consumables

KingFisher Duo		5400100
KingFisher Duo 12-tip comb	50 pieces	5012501000
KingFisher Duo elution strip	40 pieces	5012501100
DeepWell plate 2 ml KingFisher	50 pieces	5012401700

Related Products	Package Size	Catalogue No.
InviMag® Pathogen Kit /KF96	1 x 96 preparations	7450300100
InviMag® Pathogen Kit /KF96	5 x 96 preparations	7450300200
RTP® Pathogen Kit	50 preparations	1040500200
RTP® Pathogen Kit	250 preparations	1040500300
RTP® DNA/ RNA Virus Mini Kit	50 preparations	1040100200
RTP® DNA/ RNA Virus Mini Kit	250 preparations	1040100300
RTP® Bacteria DNA Mini Kit	50 preparations	1033200200
RTP® Bacteria DNA Mini Kit	250 preparations	1033200300
Invisorb® Virus RNA HTS 96 Kit/ X	4 x 96 preparations	7143310300
Invisorb® Virus RNA HTS 96 Kit/ X	24 x 96 preparations	7143310400
Invisorb® Virus DNA HTS 96 Kit/ X	4 x 96 preparations	7142310300
Invisorb® Virus DNA HTS 96 Kit/ X	24 x 96 preparations	7142310400
InviMag® Virus RNA Mini Kit/ KF 96	1 x 96 preparations	7443300100
InviMag® Virus RNA Mini Kit/ KF 96	5 x 96 preparations	7443300200
InviMag® Virus DNA Mini Kit/ KF 96	1 x 96 preparations	7442300100
InviMag® Virus DNA Mini Kit/ KF 96	5 x 96 preparations	7442300200
InviMag® Virus DNA/ RNA Mini Kit/ KFmL	15 preparations	2441150100
InviMag® Virus DNA/ RNA Mini Kit/ KFmL	75 preparations	2441150200
InviMag® Virus DNA/ RNA Mini Kit	50 preparations	1440100200
InviMag® Virus DNA/ RNA Mini Kit	250 preparations	1440100300
InviMag® Bacteria DNA Mini Kit/ KFmL	15 preparations	2433150100
InviMag® Bacteria DNA Mini Kit/ KFmL	75 preparations	2433150200

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Possible suppliers for centrifuges:

Eppendorf AG
22331 Hamburg, Germany
Phone: +49 (0) 40 53801 0
Fax: +49 (0) 40 53801 556
E-Mail: eppendorf@eppendorf.com
Internet: www.eppendorf.com

SIGMA Laborzentrifugen GmbH
37507 Osterode am Harz, Germany
Phone: +49-5522-5007-0
Fax: +49-5522-5007-12
E-Mail: info@sigma-zentrifugen.de
Internet: www.sigma-zentrifugen.de

stratec molecular

STRATEC Molecular GmbH
Robert-Rössle-Str. 10
13125 Berlin, Germany

Phone: +49 30 94 89 29 01
Fax: +49 30 94 89 29 09
E-mail: info.berlin@stratec.com

www.stratec.com

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